

Supporting Information

A time trend study of significantly elevated perfluorocarboxylate levels in humans after using fluorinated ski wax

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List of abbreviations

LOD- limit of detection

MS - mass spectrometry

PFBS - perfluorobutane sulfonate

PFBA- perfluorobutanoic acid

PFC - perfluorinated chemical

PFDA - perfluorodecanoic acid

PFHpA - perfluoroheptanoic acid

PFHxA - perfluorohexanoic acid

PFHxS - perfluorohexane sulfonate

PFNA - perfluorononanoic acid

PFOA - perfluorooctanoic acid

PFOS - perfluorooctane sulfonate

PFPeA- perfluoropentanoic acid

PFUnDA - perfluoroundecanoic acid

RRF – Relative response factor

RSD - relative standard deviation

UPLC - ultra performance liquid chromatography

Chemicals. Ammonium acetate (>99%, pa for HPLC) was purchased from Fluka (Steinheim, Germany), formic acid (98-100%) from Scharlau (Barcelona, Spain), and methanol (HPLC) from Labscan (Dublin, Ireland). All water used was laboratory produced ultra pure water. Ammonium hydroxide (25% in water) was purchased from Merck (Darmstadt, Germany). Perfluorobutane sulfonate (PFBS) tetrabutylammonium salt (> 98%), PFOS potassium salt (> 98%), perfluorodecanoic acid (PFDA; > 97%), and perfluorohexanoic acid (PFHxA, > 97%) were purchased from Fluka. Perfluoroheptanoic acid (PFHpA, 99%), PFNA, (97%), PFOA, (96%) and perfluoroundecanoic acid (PFUnDA, 95%) were purchased from Aldrich (Steinheim, Germany and Milwaukee, WI). Perfluorohexane sulfonate (PFHxS, 98%) was purchased from Interchim (Montlucon, France). Perfluorobutanoic acid (PFBA; >98%), perfluoropentanoic acid (PFPeA; >98%) and ¹³C₄-labeled PFOA, ¹³C₄-labeled PFOS and ¹³C₅-labeled PFNA were from Wellington Laboratories (Guelph, Ontario, Canada).

UPLC gradient program. A gradient program delivering mobile phases consisted of 2 mM ammonium acetate in water (A) and 2 mM ammonium acetate in methanol (B) was prepared. The gradient sets off with 60% A for 0.3 minutes followed by 5 minutes ramp up to 94% B followed by 1 minute wash sequence of 100% mobile phase B before the program reverts to initial conditions of 100% for three minutes allowing the system to equilibrate.

1 Table S1. Performance of the methods for extracting and analyzing whole blood samples.

Compound	Recovery % (CV%)	Detection limit ^a (ng/mL)	Method reproducibility interday n=4 (RSD%)
PFBA	89 (19%)	0.081	67
PFPeA	- ^b	0.062	123
PFHxA	92 (4%)	0.07	31
PFHpA	82 (5%)	0.369	3
PFOA	82 (8%)	0.333	1
PFNA	86 (4%)	0.102	15
PFDA	92 (14%)	0.097	20
PFUnDA	124 (25%)	0.051	14
PFBuS	77 (6%)	0.017	87
PFHxS	70 (6%)	0.296	14
PFOS	78 (4%)	0.018	11

2 ^a Not including eventual blank concentrations

3 ^b Not included as ¹³C in standard

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5 Table S2. Individual levels of perfluorinated carboxylates (C4-C11) and perfluorosulfonates PFBS, PFHxS and PFOS in whole
6 blood (ng/mL) Pre-season (Sep 2007), during World-Cup season 2007/2008 (Dec 2007 - Mar 2008) and post-season (Apr - Aug
7 2008). – indicates that no sample was provided.

<i>Technician 1</i>	PFBA	PFPeA	PFBuS	PFHxA	PFHpA	PFHxS	PFOA	PFNA	PFOS	PFDA	PFUnDA
Sep -07	0.12	<0.06	0.02	<0.07	0.82	<0.30	4.80	0.86	0.28	0.87	0.11
Dec -07	0.68	0.14	<0.02	12.2	1.22	<0.30	6.28	0.76	0.34	1.06	0.14
Jan -08	0.51	<0.06	0.03	2.52	3.81	<0.30	12.4	1.29	0.30	2.27	0.20
Feb -08	0.33	<0.06	0.03	2.39	5.34	<0.30	14.3	1.82	0.31	2.21	0.39
Mar -08	0.42	<0.06	<0.02	6.21	6.60	<0.30	16.8	2.20	0.39	2.23	0.35
Apr -08	-	-	-	-	-	-	-	-	-	-	-
May -08	0.57	<0.06	<0.02	<0.07	7.29	0.91	20.1	2.81	0.36	3.72	0.46
June -08	0.16	<0.06	0.02	<0.07	5.69	0.60	16.8	1.67	0.34	1.79	0.30
July -08	-	-	-	-	-	-	-	-	-	-	-
Aug -08	-	-	-	-	-	-	-	-	-	-	-
<i>Technician 2</i>	PFBA	PFPeA	PFBuS	PFHxA	PFHpA	PFHxS	PFOA	PFNA	PFOS	PFDA	PFUnDA
Sep -07	0.09	<0.06	<0.02	<0.07	<0.37	1.93	8.54	3.84	24.4	1.90	0.18
Dec -07	0.23	<0.06	<0.02	4.84	<0.37	1.78	10.1	4.74	26.4	2.41	0.39
Jan -08	0.18	<0.06	<0.02	<0.07	2.30	2.05	14.2	4.35	25.8	3.15	0.43
Feb -08	<0.08	<0.06	<0.02	2.79	1.41	1.58	15.0	3.64	22.9	2.94	0.55
Mar -08	0.21	0.07	<0.02	1.42	2.52	2.03	19.9	5.69	24.8	3.33	0.36
Apr -08	0.07	<0.06	<0.02	0.09	1.66	4.29	21.9	6.07	23.0	3.48	0.34
May -08	0.10	<0.06	0.04	0.09	0.63	4.26	23.1	6.60	25.2	3.77	0.33
June -08	<0.08	<0.06	0.02	<0.07	<0.37	3.25	19.6	6.40	21.4	4.82	0.37
July -08	<0.08	<0.06	0.02	<0.07	<0.37	3.56	21.0	7.14	22.0	4.51	0.47
Aug -08	0.10	<0.06	0.02	<0.07	<0.37	3.79	19.3	6.05	22.2	4.12	0.31
<i>Technician 3</i>	PFBA	PFPeA	PFBuS	PFHxA	PFHpA	PFHxS	PFOA	PFNA	PFOS	PFDA	PFUnDA

<i>Sep -07</i>	0.26	<0.06	<0.02	<0.07	1.03	1.41	151	14.3	14.9	8.84	0.79
<i>Dec -07</i>	0.43	0.10	<0.02	5.4	1.54	1.14	150	17.8	13.8	12.2	1.41
<i>Jan -08</i>	0.40	<0.06	<0.02	0.33	3.22	1.18	150	15.1	12.2	13.9	1.01
<i>Feb -08</i>	0.11	0.10	<0.02	5.31	3.84	0.78	146	14.7	12.1	9.21	1.13
<i>Mar -08</i>	0.11	<0.06	<0.02	1.19	4.80	1.47	150	16.8	13.8	20.1	2.12
<i>Apr -08</i>	<0.08	<0.06	<0.02	<0.07	3.53	0.96	153	17.6	12.1	23.6	2.48
<i>May -08</i>	<0.08	<0.06	<0.02	<0.07	2.06	3.27	149	20.0	16.6	15.6	1.66
<i>June -08</i>	<0.08	<0.06	<0.02	<0.07	1.44	2.39	138	15.4	13.0	11.4	1.06
<i>July -08</i>	-	-	-	-	-	-	-	-	-	-	-
<i>Aug -08</i>	<0.08	<0.06	<0.02	<0.07	0.87	1.22	133.6	14.0	11.3	17.0	1.83

<i>Technician 4</i>	PFBA	PFPeA	PFBuS	PFHxA	PFHpA	PFHxS	PFOA	PFNA	PFOS	PFDA	PFUnDA
<i>Sep -07</i>	0.11	<0.06	0.03	<0.07	0.76	1.70	127	20.9	12.7	6.83	1.04
<i>Dec -07</i>	0.20	<0.06	0.02	2.23	1.30	1.90	131	20.5	12.2	6.65	0.73
<i>Jan -08</i>	0.32	<0.06	<0.02	0.33	3.65	1.71	125	20.5	12.2	6.82	1.01
<i>Feb -08</i>	0.09	<0.06	0.02	1.02	3.11	1.58	114	19.7	13.0	6.62	1.00
<i>Mar -08</i>	0.40	<0.06	<0.02	0.22	3.62	1.94	119	17.5	10.8	8.67	1.16
<i>Apr -08</i>	<0.08	<0.06	<0.02	<0.07	3.43	2.02	119	18.1	10.8	9.51	1.34
<i>May -08</i>	<0.08	<0.06	<0.02	<0.07	2.52	1.89	122	19.2	11.2	9.78	1.33
<i>June -08</i>	<0.08	<0.06	<0.02	<0.07	1.41	1.75	113	17.8	11.2	8.43	1.18
<i>July -08</i>	<0.08	<0.06	0.02	<0.07	1.16	1.84	101	17.2	10.7	8.34	1.40
<i>Aug -08</i>	<0.08	<0.06	<0.02	<0.07	1.02	1.62	109	18.9	10.2	8.19	1.30

<i>Technician 5</i>	PFBA	PFPeA	PFBuS	PFHxA	PFHpA	PFHxS	PFOA	PFNA	PFOS	PFDA	PFUnDA
<i>Sep -07</i>	0.09	0.08	<0.02	<0.07	<0.37	1.63	9.97	1.65	13.7	1.02	0.35
<i>Dec -07</i>	0.20	0.07	<0.02	2.01	0.75	1.64	11.7	1.98	14.3	1.63	0.38
<i>Jan -08</i>	0.28	0.06	<0.02	0.68	3.62	1.72	15.1	2.49	13.8	1.95	0.38

Feb -08	<0.08	<0.06	<0.02	5.17	4.80	1.80	19.7	2.76	18.6	3.15	0.75
Mar -08	0.46	<0.06	<0.02	1.74	6.27	1.54	21.5	3.68	14.9	3.65	0.65
Apr -08	-	-	-	-	-	-	-	-	-	-	-
May -08	-	-	-	-	-	-	-	-	-	-	-
June -08	-	-	-	-	-	-	-	-	-	-	-
July -08	-	-	-	-	-	-	-	-	-	-	-
Aug -08	-	-	-	-	-	-	-	-	-	-	-

Technician 6	PFBA	PFPeA	PFBuS	PFHxA	PFHpA	PFHxS	PFOA	PFNA	PFOS	PFDA	PFUnDA
Sep -07	-	-	-	-	-	-	-	-	-	-	-
Dec -07	0.49	0.14	<0.02	0.80	11.6	2.42	276	64.9	25.5	22.1	2.84
Jan -08	-	-	-	-	-	-	-	-	-	-	-
Feb -08	0.38	<0.06	<0.02	0.34	12.1	2.40	260	59.8	25.9	19.6	2.39
Mar -08	1.08	0.12	<0.02	0.27	11.6	1.99	253	55.6	24.0	21.9	2.82
Apr -08	<0.08	<0.06	0.02	0.08	10.2	1.32	268	56.9	26.7	9.05	1.19
May -08	-	-	-	-	-	-	-	-	-	-	-
June -08	<0.08	<0.06	0.02	<0.07	6.88	1.24	249	44.6	22.0	10.3	1.22
July -08	-	-	-	-	-	-	-	-	-	-	-
Aug -08	-	-	-	-	-	-	-	-	-	-	-

Technician 7	PFBA	PFPeA	PFBuS	PFHxA	PFHpA	PFHxS	PFOA	PFNA	PFOS	PFDA	PFUnDA
Sep -07	-	-	-	-	-	-	-	-	-	-	-
Dec -07	0.28	0.07	<0.02	0.65	0.67	0.77	99.9	10.1	8.23	6.12	0.60
Jan -08	-	-	-	-	-	-	-	-	-	-	-
Feb -08	0.12	<0.06	<0.02	0.14	2.39	0.69	106	12.2	8.11	7.32	0.67
Mar -08	0.32	<0.06	<0.02	0.15	2.23	0.82	104	11.8	8.14	7.74	0.73
Apr -08	-	-	-	-	-	-	-	-	-	-	-

<i>May -08</i>	-	-	-	-	-	-	-	-	-	-	-
<i>June -08</i>	-	-	-	-	-	-	-	-	-	-	-
<i>July -08</i>	-	-	-	-	-	-	-	-	-	-	-
<i>Aug -08</i>	-	-	-	-	-	-	-	-	-	-	-
Technician 8	PFBA	PFPeA	PFBuS	PFHxA	PFHpA	PFHxS	PFOA	PFNA	PFOS	PFDA	PFUnDA
<i>Sep -07</i>	0.13	0.09	<0.02	0.13	19.3	1.35	474	145	7.17	10.8	2.19
<i>Dec -07</i>	0.58	<0.06	<0.02	0.71	19.8	1.21	528	163	6.95	13.1	2.55
<i>Jan -08</i>	-	-	-	-	-	-	-	-	-	-	-
<i>Feb -08</i>	<0.08	<0.06	<0.02	0.14	15.1	1.57	535	133	8.00	10.2	1.35
<i>Mar -08</i>	-	-	-	-	-	-	-	-	-	-	-
<i>Apr -08</i>	<0.08	<0.06	<0.02	0.30	12.4	1.18	501	128	7.00	12.0	1.82
<i>May -08</i>	<0.08	<0.06	<0.02	0.08	11.6	1.79	520	124	9.00	10.2	0.85
<i>June -08</i>	<0.08	<0.06	<0.02	<0.07	8.99	1.87	471	104	8.04	7.29	0.58
<i>July -08</i>	<0.08	<0.06	<0.02	<0.07	7.66	1.65	468	121	8.94	8.77	1.55
<i>Aug -08</i>	-	-	-	-	-	-	-	-	-	-	-

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10 Table S3. Spearmans rank correlation test (95%CI) for correlation between concentrations of PFCs in blood from professional wax
 11 technicians and their biological age.

	Number of XY pairs	Spearman, <i>r</i>	95%CI	P value (two-tailed)	Is the correlation significant? (alpha=0.05)
PFBA	56	-0.3574	-0.572 to -0.096	0.0069	Yes
PFPeA	56	-0.1675	-0.419 to 0.108	0.2174	No

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PFBuS	56	0.0173	-0.254 to 0.286	0.8993	No	12
PFHxA	56	-0.1641	-0.416 to 0.111	0.2268	No	
PFHpA	56	-0.0319	-0.2997 to 0.241	0.8156	No	13
PFHxS	56	0.6923	0.519 to 0.811	P<0.0001	Yes	14
PFOA	56	0.2601	-0.0111 to 0.496	0.0529	No	
PFNA	56	0.4379	0.190 to 0.633	0.0007	Yes	15
PFOS	56	0.5945	0.386 to 0.745	P<0.0001	Yes	16
PFDA	56	0.2065	-0.0676 to 0.452	0.1268	No	
PFUnDA	56	0.154	-0.121 to 0.407	0.2572	No	17

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19 Table S4. Spearmans rank correlation coefficient, r , between concentrations of different perfluorinated compounds in whole blood
 20 samples from men working as professional ski wax technicians (n=8). ^a P0.01-0.05 ^b P<0.001.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFBuS	PFHxS
PFPeA	0.713 ^b									
PFHxA	0.603 ^b	0.428 ^b								
PFHpA	0.119	-0.034	0.217							
PFOA	-0.258	-0.264 ^a	-0.155	0.607 ^b						
PFNA	-0.326 ^a	-0.250	-0.213	0.495 ^b	0.924 ^b					
PFDA	-0.202	-0.225	-0.216	0.433 ^b	0.890 ^b	0.809 ^b				
PFUnDA	-0.174	-0.150	-0.146	0.491 ^b	0.831 ^b	0.812 ^b	0.914 ^b			
PFBuS	-0.083	-0.069	-0.085	-0.099	-0.102	-0.073	-0.179	-0.240		
PFHxS	-0.309 ^a	-0.198	-0.287	-0.222	0.058	0.228	0.112	0.060	-0.005	
PFOS	-0.102	-0.044	0.043	-0.259	-0.041	0.018	0.071	0.025	-0.048	0.668 ^b

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